ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/123.293

Volume 123, pp. 293–299 *January–March 2013*

Typification of *Tuber formosanum* (*Tuberaceae, Pezizales, Ascomycota*) from Taiwan, China

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Abstract—When Hu described *Tuber formosanum* in 1992 as a new species based on collections from Dung-Pu, Nantou County, Taiwan, he neglected to designate a type, so that the name was not validly published. The current authors re-describe, illustrate, and validate *T. formosanum* based on a type selected from recent collections from the same region. *Tuber formosanum* is closely related to the *indicum* complex but differs in morphological, phylogenetic, and other respects and should be treated as a separate taxon.

Key Words—truffle, taxonomy, ITS

Introduction

A few ascocarps of *Tuber* species were collected from the natural forest of *Cyclobalanopsis glauca* (Thunb.) Oerst. (*Fagaceae*) in Dung-Pu, Nantou County, Taiwan, in late October 1987, mid-December 1988, early February 1990, and early January 1991. They grew in calcareous soils (pH 7.0) under *Cyclobalanopsis glauca* trees at 1300 m elevation. Hu (1992) described *Tuber formosanum* as a new species based on these collections. Because Hu did not designate a type in his description, the new species was not validly published (McNeill et al. 2006: Art. 37.1). Recent phylogenetic and morphological analyses of *T. formosanum* collections confirm that *T. formosanum* is an independent species, although closely related to the *T. indicum* complex. In addition, it has different host plants and is restricted to Taiwan. Consequently, *T. formosanum* should be treated as a separate taxon (Huang et al. 2009). In this paper, we re-describe,

Plate 1. Map of Taiwan showing where the type of *Tuber formosanum* was collected [adapted from Huang et al. 2009].

illustrate, and validate *T. formosanum* based on a type newly selected from specimens collected from the Ho-She district of National Taiwan University Experimental *C. glauca* Plantation, Nantou County, Taiwan. The plantation was established with trees inoculated by *T. formosanum* from the natural *C. glauca* forests in Dung-Pu, Nantou County. The distance between Ho-She plantation (23°35′01″N 120°51′36″E) and the original natural *C. glauca* forest in Dung-Pu (23°30′27″N 120°53′45″E) is 9.21 km (Pl.1).

Table 1: The ITS sequences of *Tuber* species used in this study.

Taxon	Voucher or code	Geographical origin	GenBank
T. melanosporum	A71	Spain	AF106877
	Tm13	Vaucluse, France	AF132501
	MelW	Unknown	AF300825
T. sinense	Tsin-hd01	Huidong, Sichuan, China	DQ375526
	Tsin-hd02	Huidong, Sichuan, China	DQ375527
	KUN–HKAS44319	Huidong, Sichuan, China	GU979061
	IFS89925	Huidong, Sichuan, China	GU979062
T. indicum	KUN–HKAS44999	Kunming, Yunnan, China	GU979066
	KUN–HKAS49746	Yimen, Yunnan, China	GU979067
	KUN–HKAS41312	Haikou, Yunnan, China	GU979069
	KUN–HKAS30261	Panzhihua, Sichuan, China	GU979050
	KUN–HKAS30262	Panzhihua, Sichuan, China	GU979051
T. formosanum	KUN–HKAS48268	Ho-She, Nantou, Taiwan	GU979048
	KUN–HKAS49707	Ho-She, Nantou, Taiwan	GU979049
	KUN–HKAS62628 (holotype)	Ho-She, Nantou, Taiwan	JN655530
T. aestivum	E5	Molise, Italy	AF516791

Materials & methods

The macroscopic and microscopic characters were described based on the type selected from new specimens collected from Nantou County, Taiwan in 2005. The methods followed those of Yang & Zhang (2003). Sections were made with a razor blade, mounted in a 5% KOH solution and examined under a Nikon E400 microscope. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto doubled-sided tape, mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a JEOL, JMS-5600LV SEM. The new specimens were deposited at the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN–HKAS).

DNA was extracted from ascomata using a modified CTAB protocol (Doyle 1987). The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the rDNA ITS region. PCR reaction solution and cycling parameters used by Chen & Liu (2007) were adopted and modified when necessary. The PCR reaction was performed on a Takara TP100 thermal cycler. Amplification products were electrophoresed on a 1% agarose gel, and purified with Sangon's purification kit. Sequencing was performed with a BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL automatic sequencer. Sixteen partial internal transcribed spacer ribosomal DNA (ITS-rDNA) sequences of *Tuber* species, *T. formosanum, T. melanosporum*, *T. sinense*, *T. indicum* and *T. aestivum* were used for analysis. *Tuber aestivum* was selected as outgroup (Table 1). Software and methods used in sequence alignment and phylogenetic analysis followed Chen & Liu (2007).

Taxonomy

Tuber formosanum H.T. Hu & Y. Wang, **sp. nov.** PLATE 2

MYCOBANK MB 563693

"*Tuber formosanum*" H.T. Hu, Quart. J. Exp. Forest Nat. Taiwan Univ. 6: 79–86 (1992), nom. inval. [No holotype designated].

Differs from *T. indicum* by its asci with short stipitate and spiny-reticulate ascospores, and its association with *Cyclobalanopsis glauca*.

TYPE: Taiwan, China: Nantou County, Ho-She, (E120°51′, N23°35′), in a *Cyclobalanopsis glauca* plantation, alt. 1200 m, 2005, H.D. Hu (**Holotype,** KUN–HKAS 62628; GenBank , JN655530).

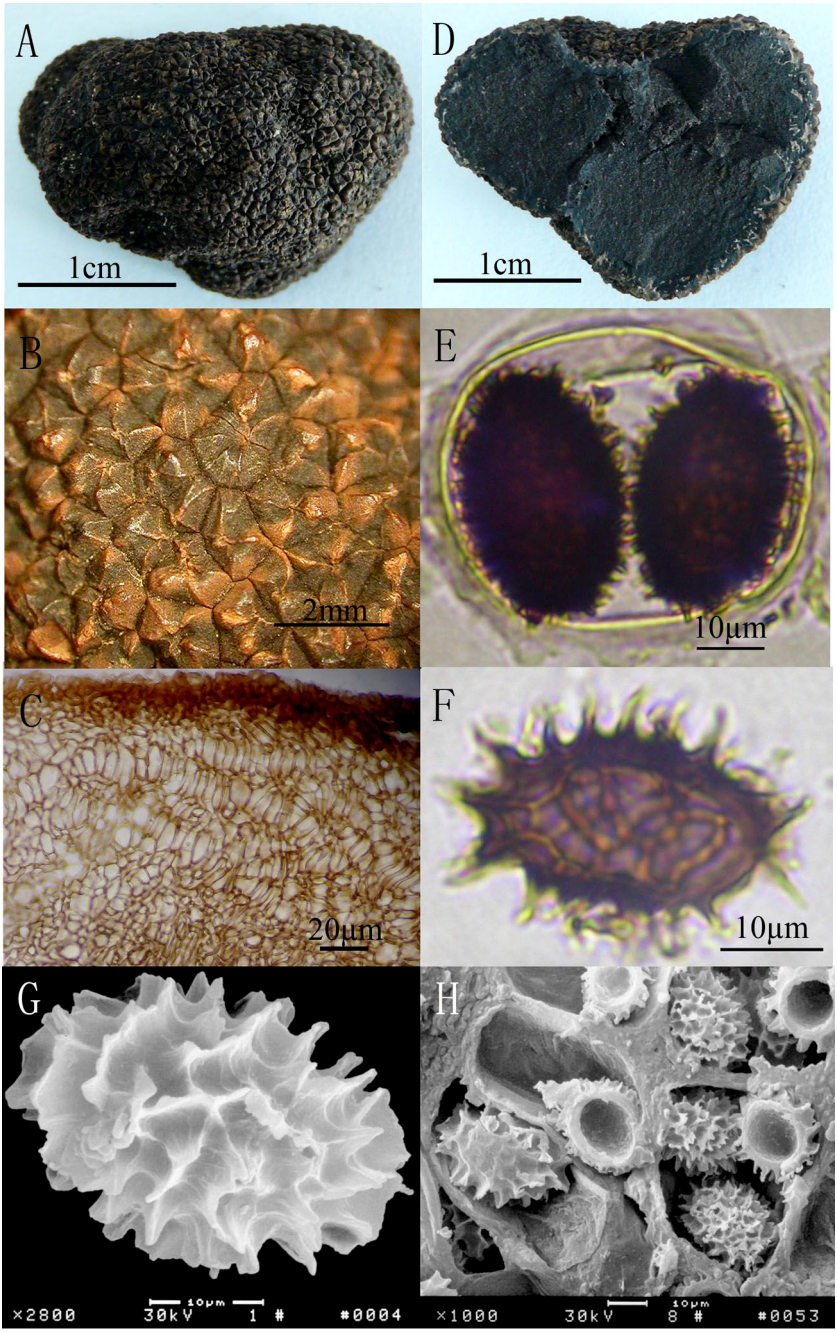
ETYMOLOGY: from the Latin *formosanum* referring to Taiwan, the location of the type collection.

ASCOMATA globose to subglobose and slightly lobed, firm, dark reddish brown to dark grayish brown, up to 8 cm in diameter, with low pyramidal warts, pentagonal, having 4–5 ridges, up to 1 mm high. ODOR slightly aromatic when mature. PERIDIUM 215–815 µm thick, variable, pseudoparenchymatous, composed of two layers: outer layer (50–)135–300(–465) µm thick, composed of angular or irregular cells (4–)8–39(–50) × (4–)5–15(–20) µm in diam., with thick dark brown walls of 0.5–2 µm; inner layer (50–)100–135(–560) µm thick, composed of hyaline to yellowish, narrow polygonal cells, merging with glebal tissue of interwoven hyphae. GLEBA solid, whitish, light yellowish when young, becoming brown to dark purplish brown at maturity, marbled with distinct, whitish, meandering veins merging at many points and sometimes penetrating through the peridium. ASCI globose or broadly elliptic, (37–)45–65(–85) × (26–)30–55(–69) µm (n = 160), with short stipitate, 1–4(–5) spored, randomly dispersed in glebal tissue. ASCOSPORES ellipsoid, whitish to yellowish when young, becoming dark brown at maturity, (27–)29–45(–48) × 20–32(–35) µm (1-spored), (26–)27–36(–39) × (18–)19–24(–28) µm (2-spored), 24–34 × (16–)18–23(–25) µm (3-spored), (25–)26–32(–33) × (17–)18–22 µm (4-spored) (excluding ornamentation, n = 40), Q = (1.17–)1.27–1.62(–1.70) (n = 170), somewhat spiny-reticulate, spines up to 2–5(–6) µm (n = 170) tall, wall (2–)3–5 (–6) µm (n = 160) thick.

ECOLOGY & DISTRIBUTION: Hypogeous in calcareous soils with pH 7.0 under *C. glauca* trees at 1200–1300 m a.s.l., fruiting from late October to early February. Known only from Nantou County, Taiwan, China.

ADDITIONAL SPECIMENS EXAMINED: **TAIWAN, CHINA. NANTOU COUNTY,** Ho-She, 23°35′N 120°51′E, in *Cyclobalanopsis glauca* plantation, alt. 1200 m, 2005, HD. Hu (KUN–HKAS48268, KUN–HKAS49707).

PLATE 2. *Tuber formosanum* (holotype, KUN–HKAS62628). A. Ascoma; B. Warts on peridium surface; C. Part of a section of peridium; D. Sectioned ascoma showing the gleba; E. Ascus with two ascospores inside; F. Ascospore showing the ornamentation; G, H. Scanning microscopic photos of ascospores (bars = 10µm).


A
1cm
D
1cm
B
2mm
E
10μm
C
20μm
F
10μm
G
×2800 30kV 1 # #0004
H
×1000 30kV 8 # #0053

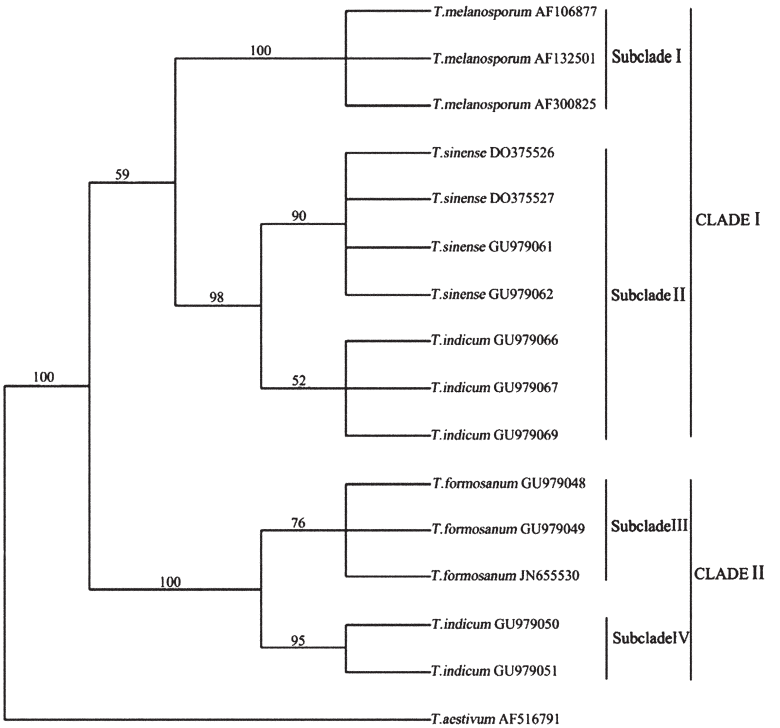


Plate 3: Strict consensus of equally parsimonious trees derived from the analysis of ITS1/5.8S/ITS2 sequence data (Length = 259steps; CI = 0.942; RI = 0.964). Numbers above branches indicate bootstrap support above 50%.

Phylogenetic analysis

The ITS phylogenetic tree revealed two major clades. Clade I divided into two subclades: subclade I corresponded to *T. melanosporum* and subclade II to *T. indicum* from Huidong, Sichuan and Yunnan prov. with high bootstrap support (≥98%). Clade II also divided into two subclades; subclade III corresponded to *T. formosanum* and subclade IV to two *T. indicum* isolates from Panzhihua, Sichuan prov. The three sequenced specimens of *T. formosanum* formed a subclade with bootstrap support of 76% (Pl.3).

Discussion

The Taiwanese truffle *Tuber formosanum* is closely related to other Asian black truffle species, such as *T. indicum* Cooke & Massee and *T. sinense* K. Tao & B. Liu (Chen 2007; Huang et al. 2009; Chen et al. 2011). However, recent phylogenetic and morphological analyses of these taxa show that although *T. formosanum* is closely related to *T. indicum* and *T. sinense*, it should still be

treated as a distinct species indigenous to Taiwan (Huang et al. 2009; Chen et al. 2011; Kinoshita et al. 2011). *Tuber formosanum* differs from *T. indicum* by its asci with short-stipitate spiny-reticulate ascospores. Most *T. indicum* asci lack stipes and its ascospore surfaces are more spike-like. *Tuber formosanum* associates naturally only with *Cyclobalanopsis glauca,* while *T. indicum* associates with both coniferous and broad-leaved trees, such as pine and chestnut. Because the original type specimen on which *T. formosanum* was based does not exist, we designate as type KUN–HKAS62628 from among specimens newly collected at the Ho-She district of National Taiwan University Experimental *C. glauca* Plantation, Nantou County, Taiwan. The plantation was established with the trees inoculated by *T. formosanum* gathered from the natural forests in Dung-Pu, Nantou County, Taiwan. The results of morphological and molecular analyses of samples from KUN–HKAS62628, KUN–HKAS48268 and KUN–HKAS49707 (Plate 3) were identical to those of Huang et al. (2009).

Acknowledgements

The authors are grateful for the help of Dr. Shannon Birch and Dr. Ian R. Hall, who critically reviewed the manuscript and provided invaluable suggestions. This study was financed by the Yunnan Program of Innovation to strong provinces by Science & Technology (No. 2009AC013), the National Science Foundation of China (No.30470011 and 31270075), the Joint Funds of the National Science Foundation of China and Yunnan Province Government (No.U0836604 and U1202262), and Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences (No.0806361121 and Y0205111L1), as well as the Knowledge Innovation Program of the Chinese Academy of Sciences (No.KSCX2-YW-G-025), and The New Zealand Institute for Plant & Food Research Ltd.

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